

SI Appendix

Model description

Abbreviations

13BPGA	1,3-bisphosphoglycerate
2PGA	2-phosphoglycerate
3PGA	3-phosphoglycerate
AK	adenylate kinase
ALD	aldolase
c	cytosolic
DHAP	dihydroxyacetone phosphate
ENO	enolase
Fru16BP	fructose 1,6-bisphosphate
Fru6P	fructose 6-phosphate
g	glycosomal
G3PDH	glycerol-3-phosphate dehydrogenase
GAP	glyceraldehyde 3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
Glc	glucose
Glc6P	glucose 6-phosphate
Gly3P	glycerol 3-phosphate
GK	glycerol kinase
GPO	glycerol-3-phosphate oxidase (=mitochondrial G3PDH plus trypanosome alternative oxidase)
HXK	hexokinase
K_{eq}	equilibrium constant
K_i	inhibition constant
K_m	Michaelis constant
PEP	phosphoenolpyruvate
PFK	phosphofructokinase
PGAM	phosphoglycerate mutase
PGI	glucose-6-phosphate isomerase
PGK	phosphoglycerate kinase
PYK	pyruvate kinase
Pyrtrans	pyruvate transport
r_v	ratio V_{max} reverse/ V_{max} forward
THT	trypanosome hexose transporter
TIM	triosephosphate isomerase
tot	total
v	enzyme rate
V	volume
V_{max}	maximum enzyme velocity

Mathematical modeling in this paper

The model with the glycosome has been described in (1) and is reproduced below. For the model without a glycosome, the original model was modified as follows:

- a. There is only a single pool of ATP, ADP and AMP, rather than distinct glycosomal and cytosolic pools.
- b. There is no conserved sum of bound phosphates
- c. The same amounts of the same enzymes are in the total cell volume rather than in the cytosol or in the glycosome.

This led to the following balance equations:

$$\frac{d[\text{Glc}]_{\text{in}}}{dt} = \frac{v_{\text{glucose transport}} - v_{\text{HXK}}}{V_{\text{tot}}} \text{ (eq. 1)}$$

$$\frac{d[\text{Glc6P}]}{dt} = \frac{v_{\text{HXK}} - v_{\text{PGI}}}{V_{\text{tot}}} \text{ (eq. 2)}$$

$$\frac{d[\text{Fru6P}]}{dt} = \frac{v_{\text{PGI}} - v_{\text{PFK}}}{V_{\text{tot}}} \text{ (eq. 3)}$$

$$\frac{d[\text{Fru16BP}]}{dt} = \frac{v_{\text{PFK}} - v_{\text{ALD}}}{V_{\text{tot}}} \text{ (eq. 4)}$$

$$\frac{d[\text{DHAP}]}{dt} = \frac{v_{\text{ALD}} + v_{\text{AOX}} - v_{\text{TIM}} - v_{\text{G3PDH}}}{V_{\text{tot}}} \text{ (eq. 5)}$$

$$\frac{d[\text{Gly3P}]}{dt} = \frac{v_{\text{G3PDH}} - v_{\text{GK}} - v_{\text{AOX}}}{V_{\text{tot}}} \text{ (eq. 6)}$$

$$\frac{d[\text{GAP}]}{dt} = \frac{v_{\text{ALD}} + v_{\text{TIM}} - v_{\text{PGK}}}{V_{\text{tot}}} \text{ (eq. 7)}$$

$$\frac{d[\text{13BPGA}]}{dt} = \frac{v_{\text{GAPDH}} - v_{\text{PGK}}}{V_{\text{tot}}} \text{ (eq. 8)}$$

$$\frac{d[\text{2PGA}]}{dt} = \frac{v_{\text{PGAM}} - v_{\text{ENO}}}{V_{\text{tot}}} \text{ (eq. 9)}$$

$$\frac{d[\text{3PGA}]}{dt} = \frac{v_{\text{PGK}} - v_{\text{PGAM}}}{V_{\text{tot}}} \text{ (eq. 10)}$$

$$\frac{d[\text{PEP}]}{dt} = \frac{v_{\text{ENO}} - v_{\text{PYK}}}{V_{\text{tot}}} \text{ (eq. 11)}$$

$$\frac{d[\text{pyruvate}]_{\text{in}}}{dt} = \frac{v_{\text{PYK}} - v_{\text{pyruvate transport}}}{V_{\text{tot}}} \text{ (eq. 12)}$$

$$\frac{d[\text{NADH}]}{dt} = \frac{v_{\text{GAPDH}} - v_{\text{G3PDH}}}{V_{\text{tot}}} \text{ (eq. 13)}$$

$$\frac{d[\text{P}]}{dt} = \frac{-v_{\text{HXK}} - v_{\text{PFK}} + v_{\text{PGK}} + v_{\text{GK}} + v_{\text{PYK}} - v_{\text{ATP utilization}}}{V_{\text{tot}}} \text{ (eq. 14)}$$

In the last equation the variable P refers to the free energy accumulation in both ATP and ADP and is defined as:

$$P \equiv 2[\text{ATP}] + [\text{ADP}] \quad (\text{eq.15})$$

The concentrations of ATP, ADP and AMP were calculated as described earlier (2), based on the conservation relation for adenine nucleotides and the equilibrium equation of adenylate kinase. The solution for ATP is:

$$[\text{ATP}] = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \quad (\text{eq. 16})$$

in which:

$$a = 1 - 4K_{\text{eq, AK}}$$

$$b = C_2 - P(1 - 4K_{\text{eq, AK}})$$

$$c = -K_{\text{eq, AK}}P^2$$

C_2 is the sum of cytosolic adenine nucleotides (4 mM). ADP was then calculated from P, ATP and equation 15 and AMP was calculated from C_2

The concentration of NAD was calculated from the conserved sum:

$$C_3 = [\text{NAD}^+] + [\text{NADH}] = 4 \text{ mM}$$

In these equations the kinetic equations of the enzymes and all parameter values were the same as in the original model ((1) and supplemental data 2). The enzyme rates v were expressed in $\text{nmol min}^{-1} (\text{mg protein})^{-1}$, the time t in min, the metabolite concentrations in mM and the total cell volume V_{tot} in $\mu\text{l} (\text{mg protein})^{-1}$.

All simulations applied to aerobic conditions, at zero concentrations of the products pyruvate and glycerol when glucose was the substrate, and at zero concentrations of glucose and pyruvate when glycerol was the substrate.

For the time simulations, the substrate was elevated from 0.01 mM to 25 mM and the concentrations of glycolytic intermediates were followed in time. When in addition to glycosome removal also the V_{max} of a kinase was lowered the substrate level was elevated from 0.1 mM to 25 mM.

Simulations were done in Jarnac 2.0 (3, 4)

Full description of the mathematical model

Below a complete description is given of the model with the glycosome present, which was published previously in:

Albert, M., Haanstra, J., Hannaert, V., Van Roy, J., Opperdoes, F., Bakker, B., and Michels, P. (2005) Experimental and in silico analyses of glycolytic flux control in bloodstream form *Trypanosoma brucei*. *The Journal of biological chemistry* **280**, 28306-28315

Rate equations

Glucose transport

$$v_{\text{THT}} = V_{\text{max}}^f \cdot \frac{[\text{Glc}]_{\text{out}} - [\text{Glc}]_{\text{in}}}{K_m^{\text{Glc}} + [\text{Glc}]_{\text{out}} + [\text{Glc}]_{\text{in}} + \alpha \cdot [\text{Glc}]_{\text{out}} \frac{[\text{Glc}]_{\text{int}}}{K_m^{\text{Glc}}}} \quad (\text{eq.1})$$

$$V_{\text{max}}^f = 108.9 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$K_m^{\text{Glc}} = 1 \text{ mM} \quad (5)$$

$$\alpha = 0.75 \quad (2)$$

Hexokinase

$$v_{\text{HXX}} = V_{\text{max}}^f \cdot \frac{\frac{[\text{Glc}]_{\text{in}}}{K_m^{\text{Glc}_{\text{in}}}} \cdot \frac{[\text{ATP}]_g}{K_m^{\text{ATP}}}}{\left(1 + \frac{[\text{ATP}]_g}{K_m^{\text{ATP}}} + \frac{[\text{ADP}]_g}{K_m^{\text{ADP}}}\right) \cdot \left(1 + \frac{[\text{Glc}]_{\text{in}}}{K_m^{\text{Glc}_{\text{in}}}} + \frac{[\text{Glc6P}]}{K_m^{\text{Glc6P}}}\right)} \quad (\text{eq. 2})$$

$$V_{\text{max}}^f = 1929 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$K_m^{\text{Glc}_{\text{in}}} = 0.1 \text{ mM} \quad (2)$$

$$K_m^{\text{ATP}} = 0.116 \text{ mM} \quad (6)$$

$$K_m^{\text{ADP}} = 0.126 \text{ mM} \quad (6)$$

$$K_m^{\text{Glc6P}} = 12 \text{ mM} \quad (7)$$

Glucose-6-phosphate isomerase

$$v_{PGI} = V_{\max}^f \cdot \frac{\frac{[Glc6P]}{K_m^{Glc6P}} - r_v \cdot \frac{[Fru6P]}{K_m^{Fru6P}}}{1 + \frac{[Glc6P]}{K_m^{Glc6P}} + \frac{[Fru6P]}{K_m^{Fru6P}}} \quad (\text{eq. 3})$$

$$V_{\max}^f = 1305 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$r_v = 1 \quad (8)$$

$$K_m^{Glc6P} = 0.4 \text{ mM} \quad (8)$$

$$K_m^{Fru6P} = 0.12 \text{ mM} \quad (8)$$

Phosphofructokinase

$$v_{PFK} = V_{\max}^f \cdot \left(\frac{K_i^1}{[Fru16BP] + K_i^1} \right) \cdot \frac{\frac{[Fru6P]}{K_m^{Fru6P}} \cdot \frac{[ATP]_g}{K_m^{ATP}}}{\left(1 + \frac{[Fru6P]}{K_m^{Fru6P}} + \frac{[Fru16BP]}{K_i^2} \right) \cdot \left(1 + \frac{[ATP]_g}{K_m^{ATP}} \right)} \quad (\text{eq. 4})$$

$$V_{\max}^f = 1708 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$K_m^{Fru6P} = 0.82 \text{ mM} \quad (9)$$

$$K_m^{ATP} = 2.6 \cdot 10^{-2} \text{ mM} \quad (9)$$

$$K_i^1 = 15.8 \text{ mM} \quad (10)$$

$$K_i^2 = 10.7 \text{ mM} \quad (10)$$

Aldolase

$$v_{ALD} = V_{\max}^f \cdot \frac{[Fru16BP] - \frac{[GAP][DHAP]_g}{K_{eq}}}{K_m^{Fru16BP} + [Fru16BP] + \frac{1}{r_v} \cdot \frac{K_m^{DHAP} [GAP]}{K_{eq}} + \frac{1}{r_v} \cdot \frac{K_m^{GAP} [DHAP]_g}{K_{eq}} + \frac{1}{r_v} \cdot \frac{[GAP][DHAP]_g}{K_{eq}} + \frac{[Fru16BP][GAP]}{K_i^{GAP}}} \quad (\text{eq. 5})$$

$$V_{\max}^f = 560 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$r_v = 1.19 \quad (11)$$

$$K_{eq} = 6.9 \cdot 10^{-2} \text{ mM} \quad (11)$$

$$K_m^{Fru16BP} = 9 \cdot 10^{-3} \cdot \left(1 + \frac{[ATP]_g}{0.68 \text{ mM}} + \frac{[ADP]_g}{1.51 \text{ mM}} + \frac{[AMP]_g}{3.65 \text{ mM}} \right) \text{ mM} \quad (\text{eq. 6})$$

$$K_m^{DHAP} = 1.5 \cdot 10^{-2} \cdot \left(1 + \frac{[ATP]_g}{0.68 \text{ mM}} + \frac{[ADP]_g}{1.51 \text{ mM}} + \frac{[AMP]_g}{3.65 \text{ mM}} \right) \text{ mM} \quad (\text{eq. 7})$$

$$K_m^{GAP} = 6.7 \cdot 10^{-2} \text{ mM} \quad (11)$$

$$K_i^{GAP} = 9.8 \cdot 10^{-2} \text{ mM (11)}$$

Triosephosphate isomerase

$$v_{TIM} = V_{\max}^f \cdot \frac{\frac{[DHAP]_g}{K_m^{DHAP}} - r_v \cdot \frac{[GAP]}{K_m^{GAP}}}{1 + \frac{[DHAP]_g}{K_m^{DHAP}} + \frac{[GAP]}{K_m^{GAP}}} \text{ (eq. 8)}$$

$$V_{\max}^f = 999.3 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (1)}$$

$$r_v = 5.7$$

$$K_m^{DHAP} = 1.2 \text{ mM (12)}$$

$$K_m^{GAP} = 0.25 \text{ mM (12)}$$

Glyceraldehyde-3-phosphate dehydrogenase

$$v_{GAPDH} = V_{\max}^f \cdot \frac{\frac{[GAP][NAD^+]}{K_m^{GAP} K_m^{NAD^+}} - r_v \cdot \frac{[13BPGA][NADH]}{K_m^{13BPGA} K_m^{NADH}}}{\left(1 + \frac{[GAP]}{K_m^{GAP}} + \frac{[13BPGA]}{K_m^{13BPGA}}\right) \cdot \left(1 + \frac{[NAD^+]}{K_m^{NAD^+}} + \frac{[NADH]}{K_m^{NADH}}\right)} \text{ (eq. 9)}$$

$$V_{\max}^f = 720.9 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (1)}$$

$$r_v = 0.67$$

$$K_m^{GAP} = 0.15 \text{ mM (13)}$$

$$K_m^{NAD^+} = 0.45 \text{ mM (13)}$$

$$K_m^{13BPGA} = 0.1 \text{ mM (13)}$$

$$K_m^{NADH} = 0.02 \text{ mM (13)}$$

Glycerol-3-phosphate dehydrogenase

$$v_{G3PDH} = V_{\max}^f \cdot \frac{\frac{[DHAP]_g [NADH]}{K_m^{DHAP} K_m^{NADH}} - r_v \cdot \frac{[Gly3P]_g [NAD^+]}{K_m^{Gly3P} K_m^{NAD^+}}}{\left(1 + \frac{[DHAP]_g}{K_m^{DHAP}} + \frac{[Gly3P]_g}{K_m^{Gly3P}}\right) \cdot \left(1 + \frac{[NADH]}{K_m^{NADH}} + \frac{[NAD^+]}{K_m^{NAD^+}}\right)} \text{ (eq. 10)}$$

$$V_{\max}^f = 465 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (1)}$$

$$r_v = 0.28 \text{ (14)}$$

$$K_m^{DHAP} = 0.1 \text{ mM (14)}$$

$$K_m^{NADH} = 0.01 \text{ mM (14)}$$

$$K_m^{Gly3P} = 2 \text{ mM (close to value in (14))}$$

$$K_m^{NAD^+} = 0.4 \text{ mM (close to value in (14))}$$

Glycerol-3-phosphate oxidase

$$v_{GPO} = V_{\max}^f \cdot \frac{\frac{[Gly3P]_c}{K_m^{Gly3P}}}{1 + \frac{[Gly3P]_c}{K_m^{Gly3P}}} \text{ (eq. 11)}$$

$$V_{\max}^f = 368 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (15)}$$

$$K_m^{Gly3P} = 1.7 \text{ mM (15)}$$

Glycerol kinase

$$v_{GK} = V_{\max}^f \cdot \frac{\frac{[Gly3P]_g}{K_m^{Gly3P}} \frac{[ADP]_g}{K_m^{ADP}} - r_v \cdot \frac{[Glycerol]}{K_m^{Glycerol}} \frac{[ATP]_g}{K_m^{ATP}}}{\left(1 + \frac{[Gly3P]_g}{K_m^{Gly3P}} + \frac{[Glycerol]}{K_m^{Glycerol}}\right) \cdot \left(1 + \frac{[ADP]_g}{K_m^{ADP}} + \frac{[ATP]_g}{K_m^{ATP}}\right)} \text{ (eq. 12)}$$

$$V_{\max}^f = 200 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (2)}$$

$$r_v = 60.86 \text{ (1, 16)}$$

$$K_m^{Gly3P} = 3.83 \text{ mM (16)}$$

$$K_m^{ADP} = 0.56 \text{ mM (16)}$$

$$K_m^{Glycerol} = 0.44 \text{ mM (16)}$$

$$K_m^{ATP} = 0.24 \text{ mM (16)}$$

Phosphoglycerate kinase

$$v_{PGK} = V_{\max}^f \cdot \frac{\frac{[13BPGA]}{K_m^{13BPGA}} \frac{[ADP]_g}{K_m^{ADP}} - r_v \cdot \frac{[3PGA]}{K_m^{3PGA}} \frac{[ATP]_g}{K_m^{ATP}}}{\left(1 + \frac{[13BPGA]}{K_m^{13BPGA}} + \frac{[3PGA]}{K_m^{3PGA}}\right) \cdot \left(1 + \frac{[ADP]_g}{K_m^{ADP}} + \frac{[ATP]_g}{K_m^{ATP}}\right)} \text{ (eq. 13)}$$

$$V_{\max}^f = 2862 \text{ nmol min}^{-1} \text{ (mg cell protein)}^{-1} \text{ (1)}$$

$$r_v = 0.47 \text{ (1, 17)}$$

$$K_m^{13BPGA} = 3 \cdot 10^{-3} \text{ mM (1, 17)}$$

$$K_m^{ADP} = 0.1 \text{ mM (18)}$$

$$K_m^{3PGA} = 1.62 \text{ mM (18)}$$

$$K_m^{ATP} = 0.29 \text{ mM} \quad (18)$$

Phosphoglycerate mutase

$$v_{PGAM} = V_{\max}^f \cdot \frac{\frac{[3PGA]}{K_m^{3PGA}} - r_v \cdot \frac{[2PGA]}{K_m^{2PGA}}}{1 + \frac{[3PGA]}{K_m^{3PGA}} + \frac{[2PGA]}{K_m^{2PGA}}} \quad (\text{eq. 14})$$

$$V_{\max}^f = 225 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$r_v = 2.2 \text{ (consistent with } K_{\text{eq}} = 0.187 \text{ mM)} \quad (1)$$

$$K_m^{3PGA} = 0.27 \text{ mM} \quad (19, 20)$$

$$K_m^{2PGA} = 0.11 \text{ mM} \quad (19, 20)$$

Enolase

$$v_{ENO} = V_{\max}^f \cdot \frac{\frac{[2PGA]}{K_m^{2PGA}} - r_v \cdot \frac{[PEP]}{K_m^{PEP}}}{1 + \frac{[2PGA]}{K_m^{2PGA}} + \frac{[PEP]}{K_m^{PEP}}} \quad (\text{eq. 15})$$

$$V_{\max}^f = 598 \text{ nmol min}^{-1} \text{ mg cell protein}^{-1} \quad (1)$$

$$r_v = 0.66 \text{ (consistent with } K_{\text{eq}} = 6.7 \text{ mM)} \quad (1)$$

$$K_m^{2PGA} = 0.054 \text{ mM} \quad (21)$$

$$K_m^{PEP} = 0.24 \text{ mM} \quad (21)$$

Pyruvate kinase

$$v_{PYK} = V_{\max}^f \cdot \frac{\left(\frac{[PEP]}{K_m^{PEP}}\right)^n \cdot \frac{[ADP]_c}{K_m^{ADP}}}{\left(1 + \left(\frac{[PEP]}{K_m^{PEP}}\right)^n\right) \cdot \left(1 + \frac{[ADP]_c}{K_m^{ADP}}\right)} \quad (\text{eq. 16})$$

$$V_{\max}^f = 1020 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \quad (1)$$

$$K_m^{PEP} = 0.34 \cdot \left(1 + \frac{[ATP]_c}{0.57 \text{ mM}} + \frac{[ADP]_c}{0.64 \text{ mM}}\right) \text{ mM} \quad (\text{eq. 17})$$

$$K_m^{ADP} = 0.114 \text{ mM} \quad (22)$$

$$n = 2.5 \quad (11, 23)$$

ATP utilisation

$$v_{ATP\text{ utilisation}} = k \cdot \frac{[ATP]_c}{[ADP]_c} \text{ (eq. 18)}$$

$$k = 50 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \text{ (2)}$$

Pyruvate transport out of the cell

$$v_{\text{Pytrans}} = V_{\text{max}}^f \cdot \frac{\frac{[Pyruvate]}{K_m^{\text{pyruvate}}}}{1 + \frac{[Pyruvate]}{K_m^{\text{pyruvate}}}} \text{ (eq. 19)}$$

$$V_{\text{max}}^f = 200 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1} \text{ (8)}$$

$$K_m^{\text{pyruvate}} = 1.96 \text{ mM (24)}$$

Differential equations

$$\frac{d[Glc]_m}{dt} = \frac{v_{THT} - v_{HXK}}{V_{tot}} \text{ (eq. 20)}$$

$$\frac{d[Glc6P]}{dt} = \frac{v_{HXK} - v_{PGI}}{V_g} \text{ (eq. 21)}$$

$$\frac{d[Fru6P]}{dt} = \frac{v_{PGI} - v_{PFK}}{V_g} \text{ (eq. 22)}$$

$$\frac{d[Fru16BP]}{dt} = \frac{v_{PFK} - v_{ALD}}{V_g} \text{ (eq. 23)}$$

$$\frac{d[GAP]}{dt} = \frac{v_{ALD} + v_{TIM} - v_{GAPDH}}{V_g} \text{ (eq. 24)}$$

$$\frac{d[DHAP]}{dt} = \frac{v_{ALD} - v_{TIM} - v_{G3PDH} + v_{GPO}}{V_{tot}} \text{ (eq. 25)}$$

in which [DHAP] represents the average DHAP concentration:

$$[DHAP] \equiv \frac{[DHAP]_g \cdot V_g + [DHAP]_c \cdot V_c}{V_{tot}} \text{ (eq. 26)}$$

$$\frac{d[13BPGA]}{dt} = \frac{v_{GAPDH} - v_{PGK}}{V_g} \text{ (eq. 27)}$$

$$\frac{d[3PGA]}{dt} = \frac{v_{PGK} - v_{PGAM}}{V_{tot}} \text{ (eq. 28)}$$

in which

$$[3PGA] \equiv \frac{[3PGA]_g \cdot V_g + [3PGA]_c \cdot V_c}{V_{tot}} \text{ (eq. 29)}$$

$$\frac{d[2PGA]}{dt} = \frac{v_{PGAM} - v_{ENO}}{V_c} \text{ (eq. 30)}$$

$$\frac{d[PEP]}{dt} = \frac{v_{ENO} - v_{PYK}}{V_c} \text{ (eq. 31)}$$

$$\frac{d[Pyruvate]}{dt} = \frac{v_{PYK} - v_{pyrtrans}}{V_c} \text{ (eq. 32)}$$

$$\frac{d[NADH]}{dt} = \frac{v_{GAPDH} - v_{G3PDH}}{V_g} \text{ (eq. 33)}$$

$$\frac{d[P]_g}{dt} = \frac{-v_{HXK} - v_{PFK} + v_{PGK} + v_{GK}}{V_g} \text{ (eq. 34)}$$

$$\frac{d[P]_c}{dt} = \frac{v_{PYK} - v_{ATP\ utilisation}}{V_c} \text{ (eq. 35)}$$

P_g and P_c denote the sums of high-energy phosphates in the glycosome and the cytosol respectively:

$$P_g \equiv 2[ATP]_g + [ADP]_g \text{ (eq. 36)}$$

$$P_c \equiv 2[ATP]_c + [ADP]_c \text{ (eq. 37)}$$

Conserved sums

$$C_1 = [AMP]_g + [ADP]_g + [ATP]_g = 6 \text{ mM (eq. 38)}$$

$$C_2 = [AMP]_c + [ADP]_c + [ATP]_c = 3.9 \text{ mM (eq. 39)}$$

$$C_3 = [NADH] + [NAD^+] = 4 \text{ mM (eq. 40)}$$

$$C_4 = [DHAP]_g + [Gly3P]_g + [Glc6P] + [Fru6P] + 2[Fru16BP] + [GAP] + [13BPGA] + P_g = 45 \text{ mM (eq. 41)}$$

$$C_5 = [DHAP]_c + [Gly3P]_c = 5 \text{ mM (eq. 42)}$$

The exchange of DHAP and Gly3P between the cytosol and glycosomes is assumed to be in equilibrium.

Calculations are done according to (8)

The $[DHAP]_c$ is calculated from

$$[DHAP]_c = \frac{C_5 \cdot [DHAP] \cdot \left(\frac{V_c}{V_g} + 1 \right)}{C_5 \cdot \frac{V_c}{V_g} + R} \text{ (eq. 43)}$$

in which

$$R \equiv [DHAP]_g + [Gly3P]_g = C_4 - [Glc6P] - [Fru6P] - 2[Fru16BP] - [GAP] - [13BPGA] - P_g \quad (\text{eq. 44})$$

[Gly3P]_c is calculated from eq. 42, [DHAP]_g from eq. 26 and [Gly3P]_g from eq. 41

The [NAD⁺] is calculated from eq. 40

The glycosomal [ATP], [ADP] and [AMP] concentrations are calculated as described in (2), based on the conserved relation for adenine nucleotides and the equilibrium constant of the adenylate kinase in that compartment:

$$[ATP]_g = \frac{-b_g + \sqrt{b_g^2 - 4a_g c_g}}{2a_g} \quad (\text{eq. 45})$$

in which

$$a_g = 1 - 4K_{eq}$$

$$b_g = C_1 - P_g (1 - 4K_{eq}^{AK})$$

$$c_g = -K_{eq} P_g^2$$

and

$$K_{eq}^{AK} = \frac{[AMP]_g [ATP]_g}{[ADP]_g^2} = 0.442 \text{ mM (25)} \quad (\text{eq. 46})$$

The concentrations of ADP and AMP are calculated from the equations for P_g and C₁ respectively. For the cytosolic concentrations [ATP]_c, [ADP]_c and [AMP]_c, P_c is substituted for P_g and C₂ by C₁.

The enzyme rates *v* are expressed in nmol min⁻¹ (mg cell protein)⁻¹, the time *t* in minutes, the metabolite concentrations in mM and the total cell volume *V*_{tot} in μl (mg protein)⁻¹.

$$V_{tot} = 5.7 \text{ } \mu\text{l (mg cell protein)}^{-1} \text{ and } \frac{V_c}{V_g} = 22.3 \quad (26)$$

Concentrations of metabolites

Glucose external: 5 mM

Glycerol: 0 mM

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